

Effect of adding wheat gluten on the quality of fermented bean curd

Master thesis in Technology

University of Turku

Department of Biochemistry

Master's Degree Programme in Food Development

May 2019

Yuqing Zhang

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin Originality Check service.

UNIVERSITY OF TURKU

Department of Biochemistry

Yuqing Zhang: Effect of adding wheat gluten on the quality of fermented bean curd

Master's Thesis of Science (Tech.), 52 p.

Food Development

May 2019

Wheat gluten is a source of high-quality protein with a wide range of sources. It has unique viscoelastic properties and high amino acid content such as glutamic acid and arginine. Fermented soybean curd is a long-established traditional food that has an important market share in condiments. In this study, the modified gluten was treated with hydrochloric acid to improve its solubility, and it was added to traditional fermented bean curd to obtain a new type of fermented bean curd. It is expected to improve umami taste and texture of the curd. For this study, use the traditional fermented bean curd as control and two treatment sample to study the effect.

An Agilent 1100 HPLC system with UV detector and ODS Hypersil column was used to analyze the free amino acids. A Thermo Scientific ISQ GC-MS system equipped with a 75 μm CAR/PDMS SPME fiber and DB-WAX (30 m \times 0.25 mm \times 0.25 μm) column was used to analyze the volatile compounds. A Fox 4000 electronic nose system equipped with Autosampler and Software Control Instruments was used to compare the difference between samples. A USPRO Colorimeter (UI-traScan Pro1166, USA) with D65 illuminant and a TA-XT plus analyzer (Perten Instruments, Hägersten, Sweden) with a cylindrical probe (diameter 50 mm) were used for the surface color and texture test.

Compared with traditional fermented bean curd, the samples added with wheat gluten showed an increase in free amino acids content, especially of umami amino acids (Glu and Asp). The analysis of volatile compounds revealed that when wheat gluten added, the volatile compounds shown a significant difference. The flavor tends to have more kinds and contents of esters, the number of esters was increased more than 10 species. According to texture profile analyze, the hardness and chewiness were decreased and the springiness and cohesiveness were increased. Treatments showed a significantly higher lightness of surface color and higher peptide contents. These changes have improved the nutritional and sensory qualities of this new product to some extent, which make it more acceptable to consumers and have broad application prospects.

Keywords: wheat gluten; fermented bean curd; volatile compounds; free amino acids

Content

1 Introduction	1
1.1 Fermented bean curd	1
1.1.1 Classification of fermented bean curd.....	1
1.1.2 Process of fermented bean curd	2
1.1.3 The nutrition of fermented bean curd.....	4
1.2 Current challenges of fermented bean curd	5
1.2.1 Impact of fermentation strains	5
1.2.2 Impact of of production process.....	7
1.2.3 Generation of flavor substances	9
1.3 Wheat gluten	12
1.3.1 The characteristic of wheat gluten	12
1.3.2 Status of application of wheat gluten	13
1.4 The modification of wheat gluten	14
1.5 Aim and purpose	17
2 Materials and methods	19
2.1 Fungal strains and spores' suspensions	19
2.2 Sufu preparation	19
2.3 Sampling for analysis	21
2.4 Physicochemical analysis	21
2.4.1 Salt content	21
2.4.2 Amino nitrogen.....	21
2.4.3 Total acid	22
2.4.4 Reducing sugar	22
2.5 Analysis of free amino acids	22
2.6 Volatile flavor substances analysis	23
2.6.1 SPME-GC-MS analysis	23
2.6.2 Electronic nose analysis	23
2.7 Color measurement.....	24

2.8	Texture profile analysis	24
2.9	Molecular weight distribution of peptides	24
2.10	Statistical analysis	24
3	Results and discussion	26
3.1	Changes in chemicals components	26
3.2	Effect of addition of gluten on sufu's color	27
3.3	Changes on free amino acids	28
3.4	Volatile components in sufu samples	31
3.5	Electronic nose analyzes	33
3.6	Molecular weight distribution of peptides in sufu	35
3.7	Texture profile analysis	37
4	Conclusion	38
5	Acknowledgements	39
6	Reference	40

1 Introduction

1.1 Fermented bean curd

1.1.1 Classification of fermented bean curd

The fermented bean curd is also called mold tofu, fermented tofu, etc.(H. L. Huang & Hesseltine, 1970). According to the Compendium of Materia Medica, a famous Chinese medicine classic, fermented bean curd is also described as "sufu". It is salted or sauced with tofu. It is considered as a kind of delicious and nutritious food, and it is enduring. The foreigners call it sufu, tosufu, toe-fu-ru, etc. It is a traditional Chinese food, dating back to thousands of years ago. As early as the 5th century AD, in the ancient books of the Northern Wei Dynasty, there is a record of the production process of fermented bean curd: "Dry bean curd and salt is matured as fermented bean curd", and the whole country has begun to produce a large amount of fermented bean curd from Ming Dynasty(Han, et al., 2001). This appetizer is common in each part of China, both north and south. It is also a seasoning for dishes such as hot pot. The famous Shaoxing fermented bean curd was even exported to Southeast Asian countries.

The fermented bean curd smells a faint smell of corruption, hence the name "sufu" (with Chinese corrupted tofu pronunciation), which is related to microbial fermentation in its production process. According to legend, the origin of the fermented bean curd was that the hawker who sold the tofu inadvertently discovered that the moldy tofu that should be corrupted was delicious, delicate and refreshing, thus, invented the fermented bean curd.

According to its appearance color and preparation process, it can be divided into red, white and grey (Figure 1). Among them, the grey one is also known as stinky tofu(P. Yan & Yi, 2012). According to the different fermentation strains, it can also be divided into mucor fermented bean curd, root mold fermented bean curd and bacterial fermented bean

curd. There are also some special varieties in the fermented bean curd, such as the one added with Lao-Chao, yellow win, sesame, sesame oil, rose, etc. The production of fermented bean curd is widely spread all over the country, and people from all over the country have formed unique local famous products according to their own tastes, such as Kedong fermented bean in the northeast, Wangzhi fermented bean curd in Beijing, Shaoxing fermented bean in Zhejiang, and Guilin fermented bean curd in Guangxi. The fermented bean curd produced in Jiangsu and Zhejiang provinces has a relatively delicate taste and slightly sweet taste. The fermented bean curd in the Bayu area is rich in flavor and spicy. The crispy fermented bean curd in Henan is more mellow and sweet(R. Wang, 2009).



Figure 1: Pictures of white, red and grey sufu

1.1.2 Process of fermented bean curd

As a traditional fermented food, fermented bean curd has been produced by natural inoculation and artificial fermentation for a long time. Until 1942, after the famous microbiologist Fang Xinfang from China isolated and purified a good strain of Wutongqiao *Mucor* from Sichuan Wutongqiao fermented bean curd, the production of fermented bean curd came to a new era of factory production(D. Zhao, 1997). There are fermented bean curd productions all over the country. Although the shapes and sizes are different, the ingredients are different, and the variety is various, the basic processing techniques are mostly the same. Nowadays, the production of fermented bean curd is purely cultivated by strains, but it is not strictly sterile in the whole production process. The participation of microorganisms in the environment gives the fermented bean curd a richer flavor, different color and texture (L. Jiang, 2012).

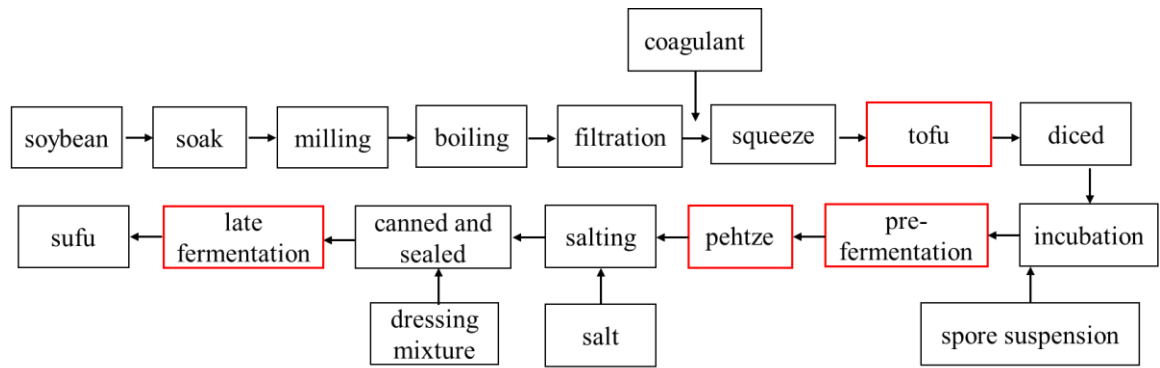


Figure 2: The process of traditional sufu

The raw material of fermented bean curd is tofu, so to produce fermented bean curd, we must first prepare the white bean curd – tofu (Figure 2). Soybean is washed and soaked, then grinded, and the soybean milk is filtered to remove the bean dregs and then boiled. The coagulant is added to the cooked soybean milk. Then molds are used to compress and the extra water is discharged to obtain the tofu. Cut the prepared white bean curd into small pieces of fixed size, place them neatly, and spray the previously cultivated microbial liquids, such as *mucor* etc., and then control the optimum growth temperature and humidity of the microorganisms for pre-fermentation. During this period, microbial produce a lot of enzymes, mainly proteases and lipases. Macromolecular nutrients such as proteins in white bean curd are decomposed into small molecules that are easy to digest and absorb, and the texture of the fermented bean curd is changed(Y. Li, 1997; Yu, et al., 2001). When the mycelial growth is strong and white, the pre-fermentation is completed.

Then enough salt is added, the excess water in the fermented bean curd is discharged, and the salt content is rapidly increased. The salt not only provides a flavor source for the fermented bean curd. Now needed the high salt content inhibits the growth of microorganisms and prevents spoilage, which is also the reason that no preservatives need to be added in the fermented bean curd. After salting, it enters the late fermentation, and according to the specific requirements of different varieties, it is added with various seasonings and wine brines. Seasonings include for example, pepper, aniseed, cinnamon, fragrant leaves or chili. After filling and sealing, it begins to ferment for one to two months. The late fermentation is the main period of the ripening of the bean curd. Small molecules such as amino acids in the fermented bean react with the alcohol and other

spices in the wine brine to form a lot of volatile esters, ketones, alcohols and other flavor substances(Cao, 2014).

1.1.3 The nutrition of fermented bean curd

The fermented bean curd is also been called as "Chinese cheese" because of high nutrition value. Its protein content is as high as 18%-20%, which is equivalent to meat and it's rich in calcium. Traditional Chinese medicine believes that fermented bean curd is sweet and warm, and has functions such as promoting blood circulation and removing blood stasis, and strengthening stomach and digestion(X. Jiang, 2016). The main source of nutrients in the fermented bean curd is of course the most primitive raw material—soybean, but the fermented bean curd has more nutritional value than the tofu, which is mainly due to the complex microbial metabolism of the fermented bean curd during the fermentation process. Thereby generating a variety of nutrients, and these metabolic activities will break down macromolecular proteins into small molecular peptides and amino acids, which is more conducive to digestion and absorption. There is increasing evidence that these small peptides have multiple physiological functions especially soybean peptides, and the fermented bean becomes a resource pool of physiologically active peptides(L. Cheng & Zhao, 2005). These peptides are to prove potential health-enhancing nutraceutical for food and pharmaceutical applications. The beneficial health effects of bioactive peptides may be several like antihypertensive, antioxidative, antiobesity, immunomodulatory, antidiabetic, hypocholesterolemic and anticancer. Soy protein is affected by cellulose, and the digestion and absorption rate are low. The direct absorption of soybeans has a protein digestion and absorption rate of only 50%; while cooked soybeans can rise to 65%; after pre-fermentation and post-fermentation in soybean curd, the digestion and absorption of protein can reach more than 92%. The microorganisms decompose the oxalic acid in the beans, which improves the utilization rate of some mineral elements such as magnesium, iron and zinc which are difficult to absorb(L. Zhang & Liu, 2002). The fermented bean curd contains no cholesterol, and the fatty acid composition is mostly unsaturated fatty acid, which helps to reduce the

cholesterol content in blood(Lu, 2005).

After microbial fermentation, it not only decomposes the original nutrients in the white bean curd, such as protein and fat, but also produces many beneficial metabolites of microorganisms, such as vitamins(He, et al., 1986). In particular, the B vitamins, riboflavin and B₁₂ are greatly increased, which can promote the metabolism of red blood cells, reduce pernicious anemia, eliminate irritability, help to concentrate and enhance memory, and promote children's growth and development(Y. Li, 1997). However, fermented bean curd is also a high-salt food, and its content of purines is also high, so it is not suitable for people with impaired renal function, gout patients, and patients with hypertension(Zhuang, et al., 2016).

Soybean is rich in soy isoflavones and is a weak estrogen(Nakamoto, et al., 2018). It is widely used in various anti-aging, anti-tumor and prevent menopausal health care products. Most of the natural soy isoflavones are in the form of glycosides. In the fermentation process of fermented bean curd, the β -glucosidic bond is opened by microbial fermentation, and the isoflavone glucoside is converted into free isoflavone aglycone, which has higher biological activity(Yin, et al., 2005).

1.2 Current challenges of fermented bean curd

1.2.1 Impact of fermentation strains

In the old days, the traditional fermented bean curd was naturally inoculated, naturally fermented, the fermentation cycle was long, the product quality was difficult to control, and even the safety was difficult to guarantee, therefore, it was gradually replaced by pure fermentation. At present, the commonly used strains of fermented bean curd are molds, yeasts and bacteria. The most widely used strains are molds (Figure 3), among which *Wutongkiao mucor*, *Furu mucor*(Y. Zhao & Zheng, 1999), *Rhizopus chinensis*, and *Rhizopus oryzae*(Kang & Lee, 2013) are most commonly used.

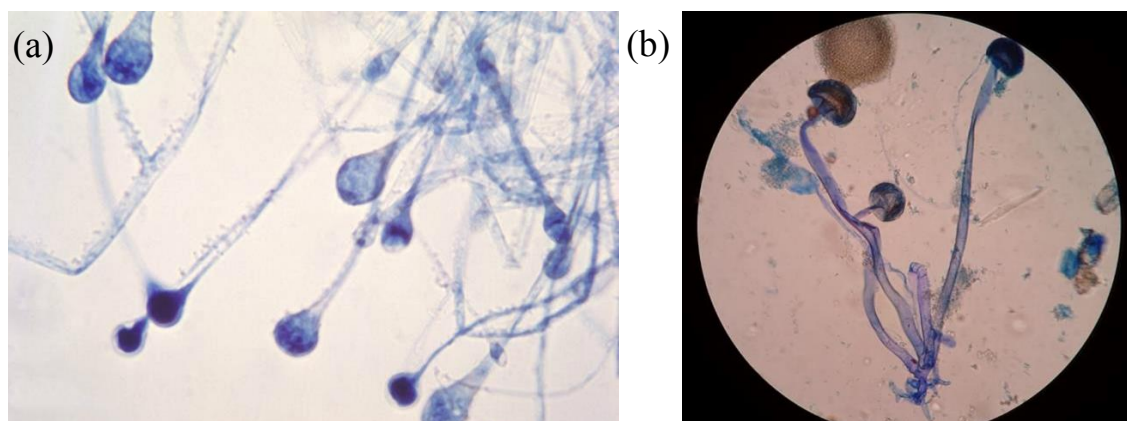


Figure 3: The most widely used strains in sufu production, microscope photos: *mucor* (a) and *Rhizopus* (b) (Image resource originated from the network. Colored by lactic acid carbonic acid blue staining solution)

Mucor has produced a variety of complex enzymes such as protease, amylase and lipase in the pre-fermentation fermentation, and has both a terminal peptidase and an endopeptidase. Therefore, the protein produced by hydrolysis has no bitter taste (J. Zhou, et al., 2007). After pre-fermentation, the *mucor* mycelium on the bean curd shows the formation of a dense film that gives the product a good appearance and helps to maintain the block shape. Xiaomiao Luo and Wang (2015) and others studied the multi-strain compound fermented bean curd, and prepared a combination of mold liquid: yeast liquid: the lactic acid bacteria liquid was a mixed starter of 9:1:1. After being cultured at 25 °C for 5 days, it was taken out and dispersed, and dried at 40 °C for 18 hours to obtain a fermented bean curd starter with good microbial activity. Ma Li et al.(L. Ma, et al., 2015) applied the space mutagenic *Mucor* ZY-3 to the production of fermented bean curd, and the obtained fermented bean curd has outstanding anti-pollution ability in the early stage of fermentation, and the product tastes delicious. The space mutagenesis *Mucor* ZY-3 strain has the characteristics of high temperature resistance, which can improve the shortage of easy-to-contamination of fermented bean curd in summer production, and make up for the vacancy of summer bean curd production. Cheng et al.(Y. Q. Cheng, et al., 2009) studied the feasibility of applying *yellow mucor* to the production of fermented bean curd. Through the effects of fermentation temperature on growth, biomass

accumulation and protease production of *yellow mucor*, the study found that *yellow mucor* grows well on the surface of fermented bean curd. During fermentation, a large amount of protease were produced at lower temperatures such as 15°C. There were almost no observable protein subunits in the late fermentation for 60 days. Therefore, they concluded that *yellow mucor* may be used as a surrogate strain for the production of fermented bean curd under low temperature conditions.

Wan et al. (Wan, et al., 2011) used *P.camemberti* strain and *Lactobacillus radiati*, which were traditionally used in cheese production, for fermented milk production, respectively, from four aspects: fermentation temperature, pH, NaCl concentration and amino acid nitrogen content. The results showed that the fermentation temperature was 30 °C, the fermentation time was 72 h, and the amino acid nitrogen content was 0.35% when the spore concentration of the strain was 1.0×10^5 CFU·mL⁻¹. Therefore, *P.camemberti* strain is a promising fermented bean curd producing strain with wide application prospects. Zhang Bin et al. (B. Zhang, et al., 2011) isolated and purified the strains in the fermented bean curd and re-dosed according to the protease activity to obtain the high-yield protease mixed fermentation broth with *Mucor*: *Rhizopus* 7:3, and optimized the optimum fermentation conditions: fermentation temperature 28°C, the salt content is 11%, the moisture content of the white bean curd is 80%, and the alcohol content of the wine is 12%. Han et al. (Han, et al., 2004) studied the changes of microbial communities in white bean curd, blank, salt bean curd and mature fermented bean curd during different stages of fermented bean curd fermentation. The research subjects included the total number of mesophilic aerobic bacteria, *Bacillus cereus*, lactic acid bacteria and Enterobacter. Bacterial spores and fungi have been found to be stable and safe in the fermented bean curd with a salt content of 8% or more and an alcohol content of 5% or more.

1.2.2 Impact of of production process

The research hotspots on the production process of fermented bean curd are currently

focused on the rapid production of fermented bean curd by enzymatic method. This method draws on the technology of enzymatic method to promote cooked cheese, omits the microbial cultivation and fermentation process in the traditional method, and directly treats the fermented white bean curd with enzyme preparation, which is greatly shortened the production cycle. Wang Yuepeng et al.(Y. Wang, et al., 2012) used two enzyme preparations to make a mixed enzyme preparation to promote the fermented bean curd in a ratio of 3:7, which verified the feasibility of enzymatically promoting the fermented bean curd, and provided a theoretical basis for subsequent research. However, the flavor of the enzymatic fermented bean curd produced by the current technology is still very different from the fermented bean curd produced by the conventional fermentation method. The main reason may be that the type of enzyme preparation used for enzymatic digestion is limited, and the enzyme system produced by microbial growth is a complex of various enzymes, and the fermentation open, and the microorganisms in the air also affect the flavor of the fermented bean curd(X. Huang, et al., 2018). Wang Jianming et al.(Xingda Lu & Wang, 2011) irradiated the fermented bean curd in the late stage of fermented bean curd. The results showed that irradiation can kill most *E. coli* and other bacteria, prolonging the storage period of the fermented bean curd, while the small number of microorganisms produced by the bean curd itself is difficult to exceed the national testing standards. The point of irradiation treatment is to make the enzyme activity during the late fermentation show a steady upward trend, which is beneficial to shorten the maturity time. Through the comparison of the production process of fermented bean curd and the production process of foreign cheese.

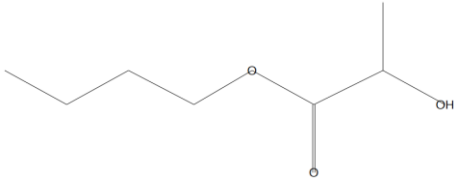
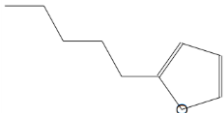

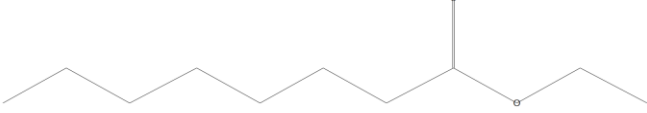

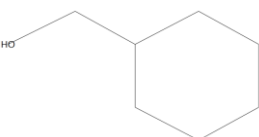
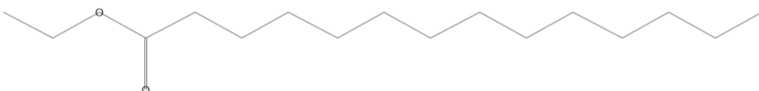
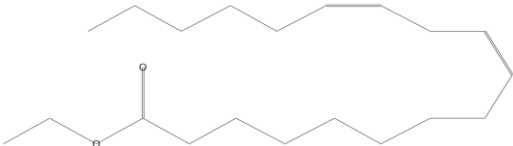
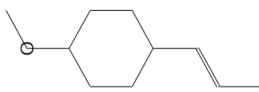
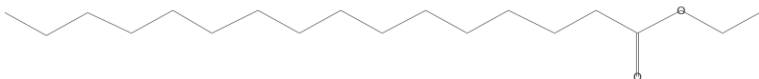
Lu Fei(Lu, 2009) analyzed the feasibility of simulating the new low-salt content of reconstituted fermented bean curd by reconstituted cheese and proposed application prospects. Ma et al.(Y.-L. Ma, et al., 2014) developed a new type of low-salt fermented bean, analyzed its quality, and studied its inhibitory activity against angiotensin-converting enzyme. The results showed that the fermentation and maturation process led to the release of peptides, which greatly improved the angiotensin transformation. The inhibitory activity of the enzyme on low-salt fermented bean curd was higher. Huang et

al.(Y.-H. Huang, et al., 2011) studied the effects of different fermentation temperatures on the content of soy isoflavones in fermented bean curd. The salt bean curd was fermented at 25°C, 35°C and 45°C for a period of 16 d. The results showed that at 45°C, the content of fermented glucosides in the condition increased most obviously, which increased from 9.78% to 40.32% compared with the content in the white bean curd; the malonyl glucoside decreased the most, compared with the content in the white bean curd, which increased from 13.17% to 39.88%. Shi et al.(Shi & Fung, 2000) studied the growth of foodborne pathogenic bacteria in fermented bean curd, involving four common foodborne pathogens: *Salmonella typhi*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7. Before the pre-fermentation of purebred strains of *Elegant radioactive mildew*, a certain amount of pathogenic bacteria mixture was inoculated, and the total number of mucor and pathogenic bacteria increased significantly after the pre-fermentation. After a month of fermentation, the total number of mucor and four pathogens fell to an undetectable level; as the late fermentation continued, the total number of bacteria decreased slightly but did not change significantly with the latter fermentation for one month. The fermentation process can effectively control common foodborne pathogenic bacteria, even if there is no sterilization in the fermentation process, it is still a safe food.

1.2.3 Generation of flavor substances

As a kind of condiment, the flavor of fermented bean curd is an important standard to measure its quality, and it also affects the price of the product to a large extent. At present, people's requirements for flavor are getting higher and higher. The flavor of fermented bean curd is mainly composed of esters and alcohols, and also aldehydes, acids, furans, ketones and so on (Table 1). At present, the detection of fermented flavor substances is mostly carried out by headspace solid phase microextraction, and qualitative and quantitative detection by gas chromatography and mass spectrometry(Y. Liu, et al., 2012).

Table 1: Chemical structure of several typical flavor substances in sufu

Compound Name	Molecular Formula	Structures
2,3-Butanediol	$C_4H_{10}O_2$	
2-Pentyl-furan	$C_9H_{14}O$	
Hexanoic acid ethyl ester	$C_8H_{16}O_2$	
Octanoic acid ethyl ester	$C_{10}H_{20}O_2$	
Benzaldehyde	C_7H_6O	
Benzyl alcohol	C_7H_8O	
Tetradecanoic acid ethyl ester	$C_{16}H_{32}O_2$	
Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	
Anethole	$C_{10}H_{12}O$	
Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	

Zhuang Yang et al. (Zhuang, et al., 2017). studied the effects of different stain on the flavor components of fermented bean curd, fermented the fermented bean curd with three

different molds, and analyzed the flavor of the pre-fermentation and mature fermented bean curd. They found that different stains had a significant effect on the flavor. And the formation of the flavor of the bean curd is mainly in the late fermentation process. Chyong-Hsyuan et al. (Hwan & Chou, 2010) compared the effect of adding ethanol on the flavor of fermented bean curd in late fermented milk. It was found that the addition of ethanol had a significant effect on the flavor of fermented bean curd. With the prolongation of fermentation in the late stage, the content of various flavor substances in the fermented bean curd slowly increased. The addition of ethanol to fermented milk produces more ester compounds.

Wang Yuepeng et al. (Y. Wang, Li, & Wang, 2012) studied the changes of flavor substances in the production process of fermented bean curd. In the white bean curd, the ester material accounted for 33.33% of the total volatile flavor substances, and the alcohol substances accounted for 14.81%. In the mature fermented bean curd, the content of esters increased to 46.67%, and the amount of alcohols decreased to 6.67%. The results show that the ester substance is the main body that constitutes the flavor of the bean curd, and the alcohol may gradually undergo esterification reaction into the ester in the later fermentation. Dou et al. (Dou, et al., 2004) studied the taste substances in five kinds of fermented bean curd, and the results showed that the taste of umami and salty taste was the most obvious. The source of the taste of the bean curd was the main umami amino acids such as glutamic acid and aspartic acid. Salty taste comes from sodium and potassium ions. Potassium and calcium ions also produce bitter taste, which may be the source of bitter taste of fermented bean curd. Xie et al. (Xie, et al., 2018) studied the flavor substances in eight kinds of commercially available fermented milk, and detected 141 volatile flavor substances, including 46 kinds of esters, 17 kinds of alcohols, 31 kinds of hydrocarbons, 13 kinds of aldehydes, and ketones. There are 7 kinds of species, 6 kinds of acids, 7 kinds of ethers and 14 kinds of other substances. The flavor composition of the eight types of fermented bean showed a difference in species and content, which led to the difference in flavor of the variety. Only five flavor substances are common to all fermented milk, namely ethanol, methanol, acetaldehyde, ethyl acetate and ethyl

octanoate. These flavors may be of similar origin and constitute the main flavor of the fermented bean curd.

1.3 Wheat gluten

1.3.1 The characteristic of wheat gluten

The earliest scientific literature on the method of extracting gluten is an article published by the Italian scientist Beccari in the eighteenth century on gluten, which proposed a method for extracting gluten from washed dough(Z. Yan, et al., 2005). Early gluten was considered as by-product of wheat starch production, is inexpensive, has a narrow application range, and is oversupply. With the deepening of research on wheat gluten and the continuous development of food processing technology, the value of this high-quality protein resource has gradually been valued by people.

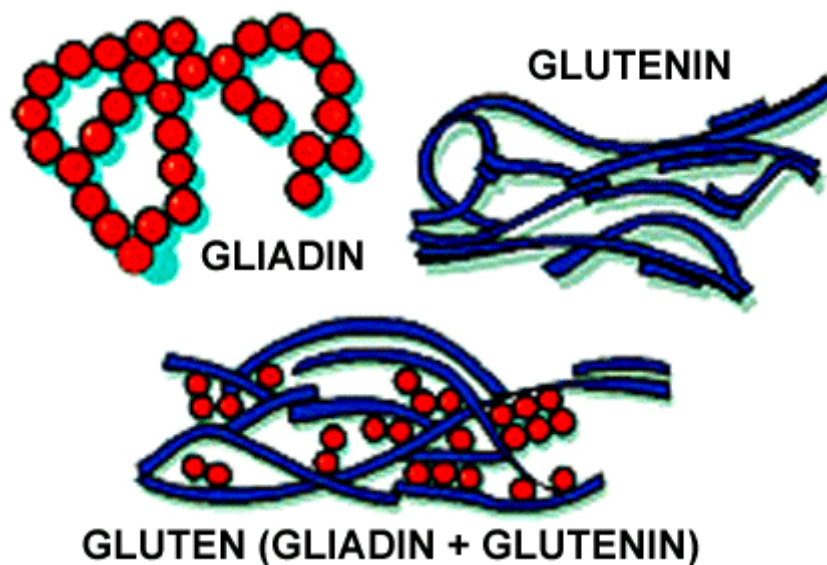


Figure 4: The component wheat gluten

The main ingredient of gluten is wheat protein, and commonly known as "gluten". The protein content in gluten is as high as 70–80%, and the composition is complex. Among the composition of wheat gluten, gliadin accounts for 40–50%, and glutenin accounts for 30–40%. The gliadin molecule is spherical and has a small molecular weight,

while the glutenin molecule is fibrous and has a large molecular weight (Figure 4). Due to the difference in the distribution of disulfide bonds, gliadin has good extensibility, while gluten is not easy to flow and has small extensibility. They are the main factors determining the rheological properties of dough (Kong & Zhou, 2003). The water absorption of gluten can form a network with a certain structure (Figure 5), which is also the root cause of its wide application in bakery products, and its film forming property, adhesiveness and emulsifying property are main functional properties.



Figure 5: The wheat gluten powder (a) and network structure (b)

The polypeptide chain forms a macromolecular polymer through disulfide bonds, and the molecular weight distribution ranges from 30,000 to tens of millions of Daltons (Da). It contains a small amount of starch, sugar, fat, cellulose and minerals (L. Wang, 2016). The gluten is rich in amino acids, including uncharged amino acid residues (such as glutamine) and non-polar amino acid residues (such as valine and leucine). While the content of charged amino acids residues is low. It is the amino acid composition that causes the gluten protein to be poorly soluble in water (S. Zhou & Zhong, 1986).

1.3.2 Status of application of wheat gluten

In the 1970s, wheat gluten was widely produced worldwide, but it was mainly produced as a by-product of wheat starch processing. By the 1980s, the annual production of gluten was only 120,000 tons (Zeng, 1989), and by the beginning of the 21st century, the global annual output had reached 1.2 million tons, the output soared 10 times, and the demand was about 900 thousand tons, the market status is still the state of "oversupply".

This is expected to continue for many years as global wheat production increases year by year. Therefore, broadening the application range of wheat gluten and improving the utilization and added value of this high-quality protein resource have become the current research hotspots.

The current application of wheat gluten is still mainly concentrated in food and related industries. Among them, the application in baking products is the most traditional and the most extensive. Wheat gluten can be used as a flour improver to extend the shelf life of baked products and improve product quality. As a special powder additive, it enriched the kinds wheat flour. As an animal feed additive, it is a source of high-quality protein and can improve the palatability of feed. When applied to convenience foods, it can improve texture characteristics of products, prevent breakage and paste phenomenon; applying to puffed foods can improve brittleness and texture. Because of its good water retention ability and emulsifying properties, gluten has recently been used in the meat industry to improve the water holding capacity and adhesion of meat(Xing & Du, 2010; F. Zhang & Wu, 2012; F. Zhu, 2016). After degraded, macromolecular aggregates of gluten are depolymerized to form many bioactive small peptides, which are also used in the health care industry to develop functional foods or vegetable protein beverages(Chen, 2010). In addition, the bio-protein membrane made of wheat gluten has good sealing and mechanical properties, and is a natural and environmentally-friendly degradable material, which can be applied to the preservation of fresh fruits and vegetables such as litchi and prolong its shelf life(S. Wang, 2000). In addition to the food industry, gluten is also used in other industries such as the industrial sector(Hu, et al., 2018; Si, 2003; L. Wang, et al., 2006). For example, a sustained release capsule wall material can be used to control the release time of the embedded material. The modified gluten powder, in which the disulfide bond is opened, can be used as a biodegradable industrial film.

1.4 The modification of wheat gluten

The biggest reason for limiting the wider application of wheat gluten is its poorly water-

soluble nature and the lack of a suitable method for dissolving macromolecular polymers in wheat gluten. For a long time, many researchers have studied this problem. At present, the modification method is widely used to depolymerize the macromolecular protein polymer to increase its solubility(Wu, et al., 1976). Commonly used methods are chemical methods, physical methods, biological methods and biochemical methods. Among them, chemical modification means acid modification and alkali modification, biochemical method is enzymatic modification, and physical modification mainly uses ultra-high pressure, extrusion, shearing and other mechanical actions or ultrasonic, radiation to change protein molecules. The spatial structure changes its properties, and the biological method uses microbial fermentation to partially hydrolyze wheat gluten, which can increase its solubility(H. Zhang, et al., 2004) .

Enzymatic modification is carried out by enzymatic preparations such as alkaline protease, trypsin and wind pepsin(K. Huang, et al., 2006). Due to its high specificity, enzymatic gluten meal produces some small peptides with physiological activity, which is gradually valued by researchers. However, due to the high cost of the enzyme preparation, and the single type of enzyme used at present, the obtained product has great limitations, and because the hydrophobic peptide content in the enzymatic hydrolysate is rich, the taste is slightly bitter, the protein emulsify ability is lowered, and the enzymatic modification needs further research and improvement(Kong, et al., 2004). Previous studies on alkaline modification found that although the alkali modification is fast, it destroys the lysine in the protein and forms lysine alanine, which indicates toxicity to mouse kidney function in toxicological studies(Tang, et al., 2006). Therefore, there are fewer subsequent studies and applications.

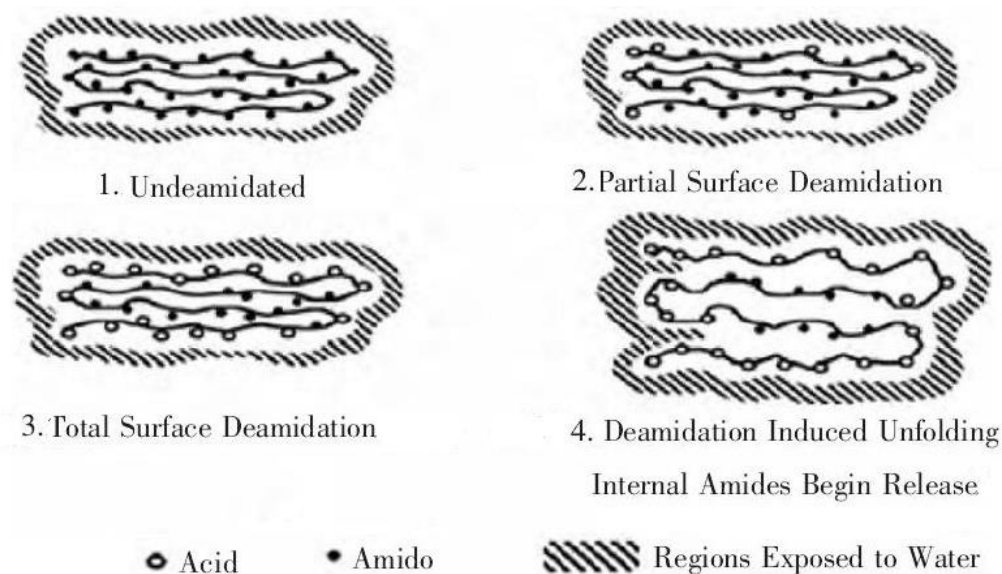


Figure 6: Exposure of amides to protein surfaces

Acid modification also has the advantage of high speed. In a weakly acidic environment, asparagine and glutamine undergo deamidation to form aspartic acid and glutamic acid. Most of the insoluble and viscous properties of gluten are usually caused by molecular associations caused by many amide groups on glutamine residues formed by hydrogen bonding (Holme, 1955; Wall, et al., 2010). Deamidation destroys this widespread hydrogen bond (Figure 6), increased the surface negative charge and electrostatic repulsion increases, thus the protein molecules expand and solubility increased. The moist hot acid treatment modified gluten can significantly improve its solubility in water, and the functional properties such as foaming and emulsifying properties are also obviously improved, and at the same time, the digestion and absorption of essential amino acids such as lysine can be promoted. Due to the wide range of sources and the modification effect is good, and hydrochloric acid is currently used (Liao, 2012). Chiu H. Wu et al. (Wu, Nakai, & Powrie, 1976) heated 5% gluten protein suspension at 121°C with 0.02 N HCl for 30 minutes or with 0.05 N HCl for 15 minutes for modification, and recovered at the isoelectric point of 4.7-4.9. It improves the emulsifying ability and stability of gluten protein greatly and is superior to the emulsifying ability and stability of soy protein isolates.

In addition, the composite modification is also a method for modifying gluten powder,

which uses the above two or more methods to achieve the desired modification effect(X. Zhao, et al., 2006).Tong Qunyi et al.(Tong, et al., 2004) used acetylation of glutamic acid powder by acetic anhydride and phosphorylation of gluten powder with sodium tripolyphosphate. The functional properties of the composite modified product obtained were modified compared with unmodified and single method. The gluten meal has been significantly improved, and the solubility of the composite modified product has increased to more than 85%.

1.5 Aim and purpose

Wheat is the main food crop in China, and the output of wheat flour is huge. Therefore, its by-product gluten raw materials are abundant and the price is low. Gluten powder is widely used in the food field and other fields due to its excellent emulsification, foaming, viscoelastic and other functional properties. At present, the application of gluten meal in the traditional field is approaching saturation, and its output is still increasing year by year, and it is in a state of oversupply for a long time. Therefore, the application range of gluten is expanded, and the utilization of this high-quality protein resource is rationally utilized. And added value has become a research hotspot.

The fermented bean curd is a traditional condiment in China. Nowadays, the food industry is developing at a high speed. The demand for food in today's society has surpassed the level of food and clothing, and it has gradually developed towards satisfying people's hobbies. The demand of texture, flavor, nutrition and safety of food are more and more high. As a traditional food, innovation is urgently needed to adapt to the trend of the times. The high salt content, unchanging flavor, long fermentation cycle, and easy breakage of traditional fermented bean curd are urgently needed to be improved through advanced technology. While enriching the variety of food products, it protects and inherits the Chinese tradition. In recent years, studies on fermented bean curd have focused on fermenting strains and fermentation mechanisms, but little research has been done on improving the quality of fermented bean from raw materials.

In this thesis, the gluten was modified by wet heat acid treatment to improve its solubility in water. Innovate in raw materials, combine it with soybeans to make a new fermented bean curd, use its rich amino acid composition and content, especially glutamine content, improve the amino acid content of fermented bean curd, improve its flavor, especially umami. Using the unique viscoelastic properties of gluten to improve its texture characteristics, enrich the variety of traditional foods such as fermented bean curd, and provide reference value for widening the application range of gluten.

This research mainly includes the following contents:

(1) Determination of the preparation process of the new type of fermented bean curd. The raw material was mixture of modified wheat gluten and soybean milk. The fermentation strain was *Wutongkiao mucor* for pre-fermentation, and finally packing the pot to carry out the post-fermentation to obtain the mature fermented bean curd.

(2) Analyze and test various physical and chemical indicators of the new type of fermented bean curd. Such as moisture content, salt content, texture parameters, surface color, amino acid nitrogen, total acid, free amino acid, sensory evaluation, volatile flavor substances, electronic nose analysis, peptide molecular weight distribution and the like. And compared them with traditional fermented bean curd.

2 Materials and methods

2.1 Fungal strains and spores' suspensions

Pure culture of *Wukongkiao Mucor* (3.25) was bought from General Microbiological Culture Collection Center (Beijing, China). The *Wukongkiao Mucor* strain was cultured on potato dextrose agar (PDA) fluid medium at 28°C for 2-3 days. The resulted spore suspension was added to a sterilized fluid medium, after static incubation at 28°C for 48 h in the thermostat incubator. Then, medium and biomass were harvested and homogenized to 10^7 mL⁻¹ spores. Prepare fresh spore suspensions before each inoculation(P. Li, et al., 2014; L. Zhu & Cai, 2014).

2.2 Sufu preparation

The process of tofu curd and sufu are shown in Figure 7. The difference between control and treatment is if it is added with acid soluble gluten. The selected soybeans were washed and soaked in triple its weight of deionised water for 10-12 h at room temperature (25°C). Then the soaked soybeans were grinded by an auto soybean milk machine (JiuYang, China). The soymilk was filtrated by 80 mesh standard sieve and the resultant okara was abandoned. The wheat gluten suspension (pre-dissolved in water) was added while boiling at 85°C for 5 min. Natural bittern whose main component was magnesium chloride and calcium chloride added to the soymilk with stirring, and sanding for half an hour. The unsolidified tofu was then pressed into the mold and extra water squeezed out. The pieces (2.0×2.0×1.5 cm) were inoculated with *Mucor* liquid (2.1.) by spraying inoculation suspension and fermented in a thermostat incubator at the 90% relative humidity at 28°C for 48 h (Z. Liu, 2000). Then get the product of pre-fermentation, pehtze. After pre-fermentation, 10% its weight of salt was added on pehtze layer by layer. The salted pehtze was transform in glass pot contained dressing mixture to ripen for 2 months at room temperature. In order to obtain sufu with 11% to 14% (w/w) salt content, the dressing mixture was added with extra salt with 5% of alcohol contents. The formula of pehtze was determined by our previous study(Yuqing Zhang, et al., 2019), the total

amount of raw material per sample was 150 g (Table 2), of which the raw material of the control sample was soybean, and the treat 1 and 2 were added with 33.3% and 42% of wheat gluten, respectively.

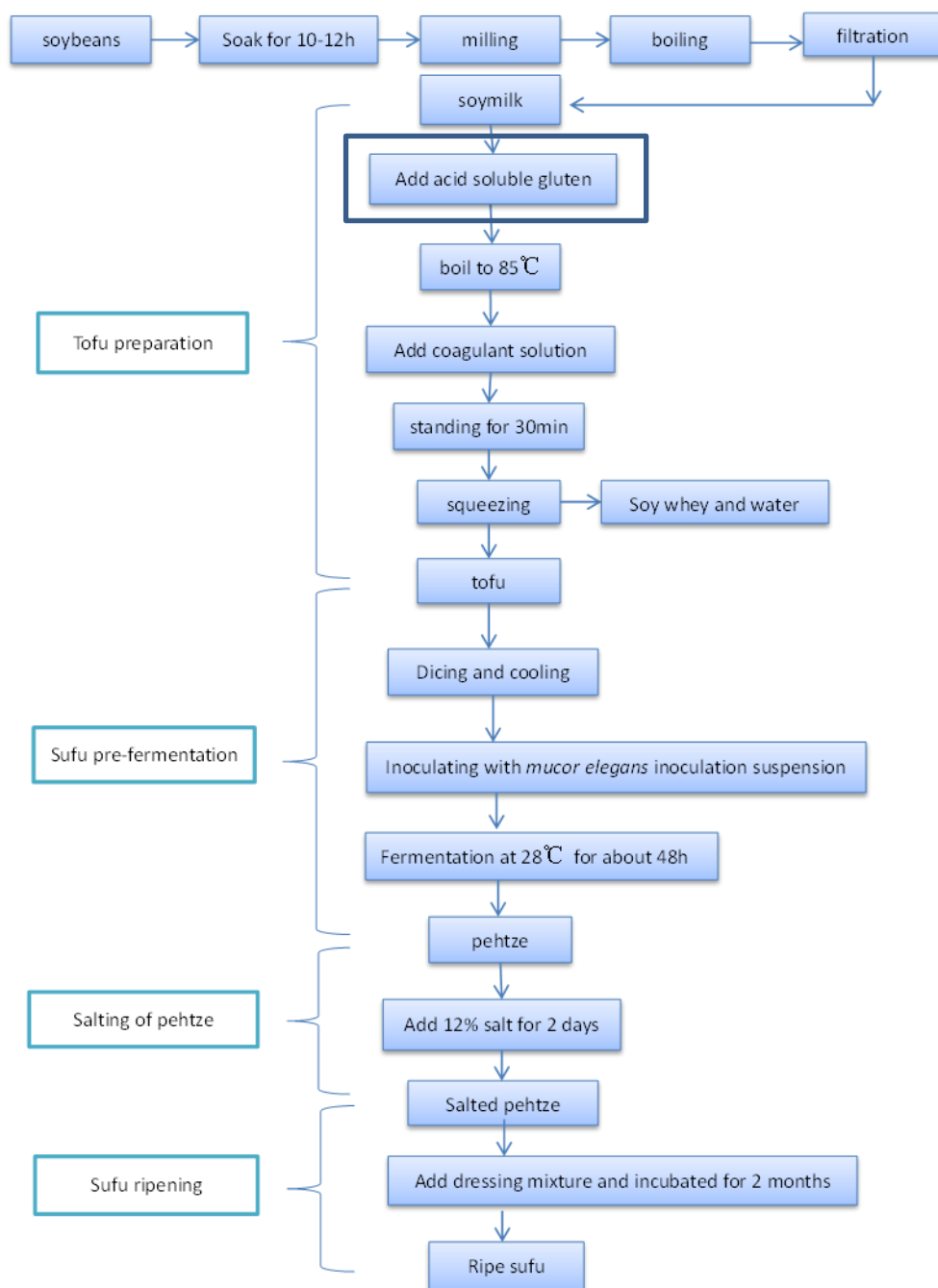


Figure 7: Basic scheme for the preparation of the fermented bean curd in the laboratory conditions
(Without adding acid soluble gluten for the control)

Table 2: Ingredients table

	Soybean/g	Gluten/g
Control	150	0
Treat 1	100	50
Treat 2	75	75

2.3 Sampling for analysis

20 g sample was weighed in a 150 mL beaker, 80 mL deionized water was added, stirred well and putted on the electric furnace, after boiling and cooling to room temperature. Then, the mixture was diluted to a 200 mL volumetric flask. Finally, the liquid was filtered to 250 mL reagent bottles with ground stopper for further use.

2.4 Physicochemical analysis

2.4.1 Salt content

The method specified in the China national standard for sufu was used (SB/10170-2007). Briefly, 2 mL of the above filtrate and 50 mL of water was added into an Erlenmeyer flask. The mixture was titrated with silver nitrate of standard titration solution with 1 mL potassium chromate as an indicator. When the solution indicated orange, the titration reaches the end point.

2.4.2 Amino nitrogen

The method used as specified in the China national standard (SB/10170-2007). Briefly, 10 mL of the sample in a 200 mL beaker with 50 mL of deionized water with gently stirred, following the mixture titrated to pH 8.2 with $0.1 \text{ mol} \cdot \text{L}^{-1}$ standard sodium hydroxide. Then the mixture was titrated to pH 9.2 after 10 mL of formalin solution was added. The volume of spent sodium hydroxide raising pH was recorded to calculate amino nitrogen content.

2.4.3 Total acid

The method used as specified in the China national standard (SB/10170-2007). The filtrates were titrated with $0.05 \text{ mol} \cdot \text{L}^{-1}$ sodium hydroxide standard solution and. The titration was terminated when the pH reached 8.2.

2.4.4 Reducing sugar

The reducing sugar was measured by DNS method according to the procedure described by Miller et al. (Miller, 1959) and Passos et al. (2018) with some modifications. To extract the reducing sugar from sufu sample, 1.5 g of sample was added 25 mL deionized water (warm-up at 50°C). The mixture was centrifuged at 3000 rpm for 10 min and the supernatant was diluted to a 25 mL volumetric flask.

The content sugars contents were estimated by the 3,5-Dinitrosalicylic acid colorimetric method. Samples were diluted twice by DNS solution. The mixture was heated in boiling water bath for 5 min and then cooled to room temperature. After cooling, the solution was made up to volume with distilled water and measured the absorbance at 540 nm. The content of reducing sugar were expressed as the average of three replicates calibrated by a standard curve.

2.5 Analysis of free amino acids

Free amino acids content in the sufu sample were analyzed using. 1 g of the sample (dry basis) was dissolved in 5% trichloroacetic acid and volume to a 25 mL volumetric flask. The mixtures were centrifuged at 10000 rpm for 10 min and filtrated through a $0.22 \mu\text{m}$ syringe filter(D. Li, et al., 2017). The filtrate was then analyzed by an Agilent 1100 HPLC system (Palo Alto, CA, USA) with UV detector and ODS Hypersil column (Thermo Fisher Scientific, Waltham, MA, USA). The column temperature was 40°C , and the column flow rate was $1.0 \text{ mL} \cdot \text{min}^{-1}$.

2.6 Volatile flavor substances analysis

2.6.1 SPME-GC-MS analysis

Samples (2.0 g) were placed in headspace vials (20 mL) with screw caps and sealed. The vials were kept warm at 50°C for 30 min to reach balance. A 75 μm CAR/PDMS SPME fiber was used for adsorption. Place it in a vial and expose it to the headspace for 40 min. A Thermo Scientific ISQ GC-MS system was used to analyze the desorbed volatiles. The conditions as follow: splitless mode; 3 min desorption time; DB-WAX (30 $\text{m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) column; He carrier gas, flow rate $1.8 \text{ mL} \cdot \text{min}^{-1}$ and detector temperature 250°C. The programmed temperature starts at 40°C and last for 3 min, $5^\circ\text{C} \cdot \text{min}^{-1}$ to 90°C, then $10^\circ\text{C} \cdot \text{min}^{-1}$ to 230°C and last for 7 min. Peaks were identified by comparison to a mass spectral database library and its retention index (RI). The mass spectrometer was operated in an electron impact (EI) ionization mode of 70 eV to obtain mass spectral data in the mass range of 35-450 $\text{m} \cdot \text{z}^{-1}$. The injection and ion source temperature were 250°C and 200°C respectively (Qi & Weng, 2008; Sun, et al., 2014).

2.6.2 Electronic nose analysis

All samples were prepared as the modified method reported by Drake (2003). (L. Zhao, et al., 2017) In brief, samples (2.0 g) were placed in headspace vials (20 mL) with screw caps and sealed. The volatile compounds were analyzed by a Fox 4000 electronic nose system (Alpha-MOS, France) equipped with Autosampler (HS-100) and Software Control Instruments (SOFTV) Repeat four times for each sample to ensure accuracy. The electronic nose parameters: clean air carrier gas and flow rate $150 \text{ mL} \cdot \text{min}^{-1}$; headspace heating 600 s; temperature 40°C and stirring speed 300 rpm. The sample headspace injection volume 1.5 mL and injection speed 1.5 mL s^{-1} . The total volume of injection needle is 5.0 mL; syringe temperature 50°C; the last data acquisition time 120 s and latency 360 s.

2.7 Color measurement

The color measurement referred to the method of Musso (Musso, et al., 2016). The difference in the color of sufufu samples was compared by measuring L^* (lightness, white-black), a^* (red-green) and b^* (yellow-blue) values using a USPRO Colorimeter (UltraScan Pro1166, USA) with D65 illuminant. Each sample randomly selected four times to determine the average. The color difference (dE^*) was calculated by Eq: $dE = (dL^{*2} + da^{*2} + db^{*2})^{1/2}$. The dL , da , and db represent the difference of L , a , b values among sufufu samples.

2.8 Texture profile analysis

The textural properties of sufufu were tested using TA-XT plus analyzer (Perten Instruments, Hägersten, Sweden) with a cylindrical probe (diameter 50 mm). The trigger force was set as 10 g. The sufufu sample was compressed to 30% at a test speed of $1 \text{ mm} \cdot \text{s}^{-1}$. The pre-test and post-test speeds were $2 \text{ mm} \cdot \text{s}^{-1}$ and $1 \text{ mm} \cdot \text{s}^{-1}$ respectively (X. Li, 2015; J. Wang, et al., 2006).

2.9 Molecular weight distribution of peptides

The molecular weight distribution of peptides was analyzed according to the method reported by Li Dandan et al. (2017). A Waters 1525 HPLC system equipped with a UV detector and TSKgel 2000 SWXL column (300 mm×7.8 mm, Tosoh Co., Tokyo, Japan) was used. The cytochrome C (12384 Da), Bacillus enzyme (1422 Da), glycine-glycine-tyrosine-arginine (451 Da) and glycine-glycine-glycine (189 Da) were used to calculate the standard calibration curves. Samples were eluted by 40% acetonitrile with 0.1% TFA. The flow rate was 0.5 mL min^{-1} and the elution was observed at 220 nm.

2.10 Statistical analysis

All experiments were performed more than three times and all data were expressed as mean \pm standard deviation. The differences among samples were analyzed by one-way

ANOVA with Duncan's test (post hoc test) using a SPSS (IBM SPSS statistics 24, USA) and $P < 0.05$ was considered as significant.

3 Results and discussion

3.1 Changes in chemicals components

Chemical compounds are playing important roles in promoting the unique tastes and flavors of fermented foods. For instances, salt can provide saltiness, sugars are sweet, and variety of amino acids can contribute to umami, sweet tastes. The results of chemicals components are shown in Table 3. The total acid and moisture content did not show a significant change.

Table 3: Contents of salt, total acid, reducing sugar, amino nitrogen and moisture in ripened sufu

Components	Total acid (g/100g)	Reducing sugar %	Amino nitrogen %	Salt %	Moisture %
control	1.38±0.37 ^{a*}	0.025±0.003 ^a	0.36±0.016 ^a	12.66±0.10 ^a	71.39±0.45 ^a
treat1	1.14±0.37 ^a	0.003±0.001 ^b	0.46±0.021 ^b	11.42±0.09 ^b	72.12±0.39 ^a
treat2	1.32±0.21 ^a	0.003±0.001 ^b	0.55±0.021 ^c	12.57±0.11 ^a	71.78±0.38 ^a

*Values were expressed as mean ± standard deviation. Repeat three times for each test.

Values in the same fraction with different letters (a-c) within a same column differ significantly ($P<0.05$).

As in some other fermented foods, such as cheese, salt plays a very important role in sufu(Han, Rombouts, & Nout, 2001). Firstly, the salt leads to the shrink of pehtze and exclusion of excess water, which makes the pehtze become harder, and the *mucor* mycelium can form a film on the surface. On the other hand, the lower water activity induced by salt prevents products from spoilage although there may be some salt tolerant bacteria such as *Clostridium* spp. and *Bacillus* spp. cause potential hazard to consumers.¹⁵ In addition, the high concentration of salt not only has ability of anti-corrosion, but also can inhibit protease activity.

Which seems interesting is that the figure of reducing sugar dropped dramatically.

The α -amylase and α -galactosidase produced during pre-fermentation hydrolyzed starch and amylose to dextrins and oligosaccharides, then the dextrins and oligosaccharides were further hydrolyzed to glucose by glucoamylase. Because wheat gluten greatly increased the relative amount of proteins, the relative amount of reducing sugar would decrease while the amino nitrogen would increase.

3.2 Effect of addition of gluten on sufu's color

For the traditional white fermented bean curd, Maillard browning occurred in late fermentation and their storage extended its color and appearance (Table 4). Previously, similar findings on the color of sufu have been reported (T. Li, et al., 2013), which revealed that at the end of the maturity period, the surface color of the sufu changes from light yellow to yellowish brown. While the differences in color between treatment groups and control become distinct. The L^* values of treatment groups were higher than control group and showed a significant difference. Both two treatment groups showed about 12% increase on the value. Besides, the control group held a much larger a^* value and lower b^* value than the treatment groups, it showed that the addition of gluten can improve the color of traditional sufu. The dE values were much greater than 6 represents significant differences visible to the human eye.

Table 4: The color of sufu samples

	L^*	a^*	b^*	dE
control	57.90±1.69 ^{a*}	4.71±0.13 ^a	20.13±0.39 ^a	46.08±1.29 ^a
treat1	64.56±2.21 ^b	3.43±0.57 ^b	19.55±0.32 ^a	40.02±1.95 ^{ab}
treat2	65.34±0.65 ^b	2.78±0.11 ^b	19.75±0.74 ^a	42.46±0.16 ^b

*Values were expressed as mean \pm standard deviation. Repeat three times for each test. Values in the same fraction with different letters (a, b) within a same column differ significantly ($P < 0.05$).

The color change observed on the sufu cube can be attributed to the results of enzymatic and non-enzymatic browning reactions that occurred during maturation.

Because of the presence of amino compounds in the dressing fluid and reducing sugar in sufu, Maillard reaction react slowly (Shibasaki & Hessbltine, 1962). Wheat gluten contains much more protein than soybean, therefore, the sufu added with wheat gluten contains relatively less sugar than the control as showed in Table 2. Although the amino acid and nitrogen contents were increased, whereas the Maillard reaction was suppressed. That may be the main reason why the treatment groups can anti-brown. While the enzymatic browning which occurs under anaerobic conditions, phenolic enzymes catalyze the formation of quinones and their polymers may also contribute to sufu browning. Because of the need of oxygen, it only occurs when the sealing conditions are weak or the package is opened.

3.3 Changes on free amino acids

The total content of FAA of the control, treat 1 and treat 2 are 1.0154, 1.5669 and 1.7086 g 100g⁻¹. For two kinds of sufu added wheat gluten, the data were whereas addition of wheat gluten significantly increased for 54.31% and 68.27% respectively compared with the control. The activated *Mucor* proteases which induce hydrolyzation of the proteins elevated the content of free amino acids during ripening. Glutamate is the predominant free amino acid in ripened sufu and which is an important considered as umami. Because wheat gluten is an abundant source of glutamate and proline, the content of glutamate and proline were increased dramatically. The total glu and asp contents were increased for 54.47% and 46.55% for treat 1 and treat 2. For the other amino acids (Figure 8), alanine, glycine, valine and serine were the main sweet amino acids. Compared with traditional fermented curd, the sweet amino acid content of gluten-added fermented bean curds also increased, but their threshold values were relatively high, so they affected the flavor of fermented bean curd less. For other hydrophobic amino acids, most of them are bitter taste. Except for the strong bitter amino acid arginine, several others' figure had increased. However, when some bitter amino acids are below their threshold, they can enhance the sweetness and umami taste of other amino acids (Xu, et al., 2017).

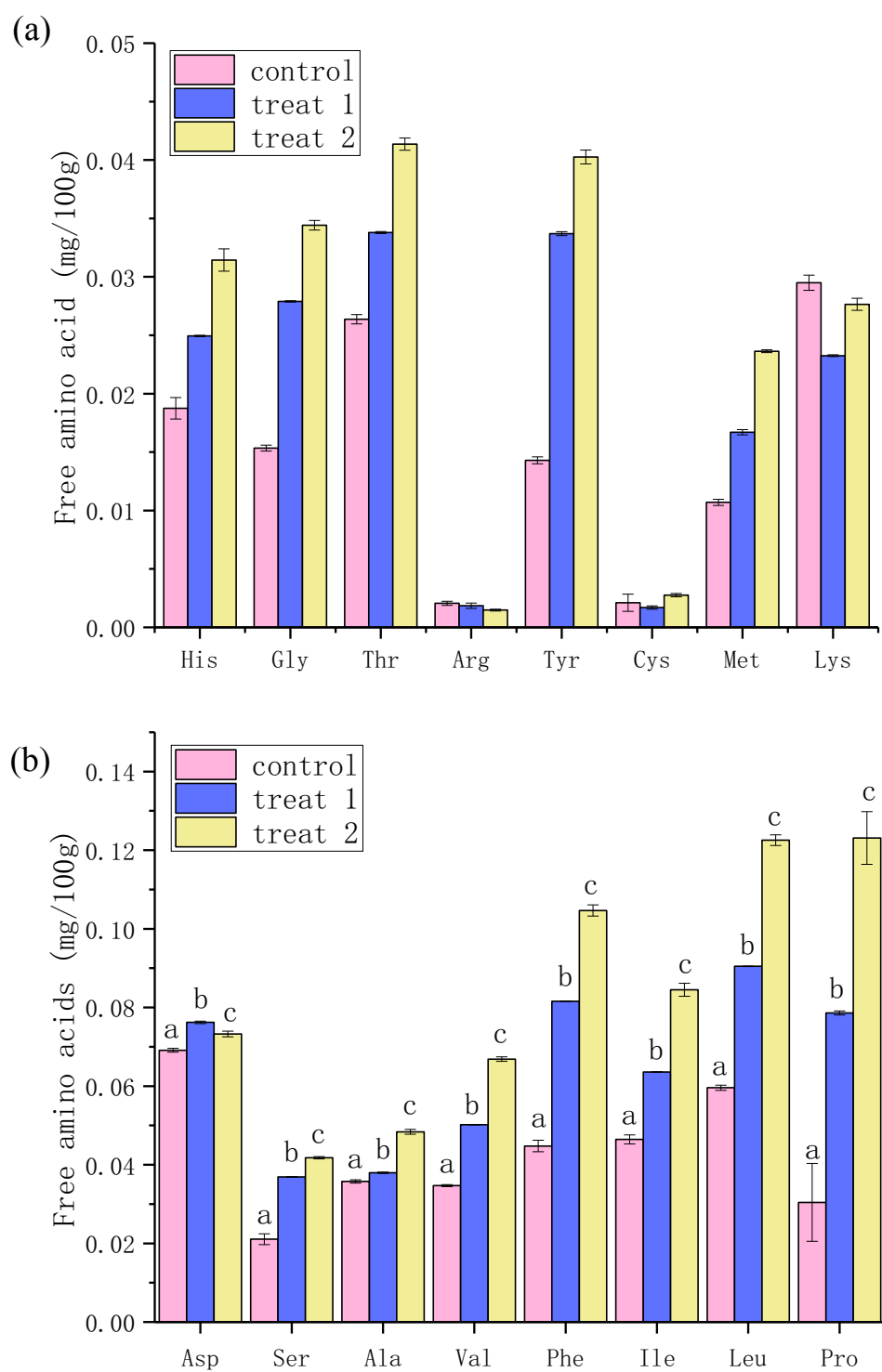


Figure 8: Free amino acid composition of three kinds of sufu after late fermentation (a&b). Different letters (a-c) on the bars indicate significant difference ($P<0.05$). Obtained by HPLC with external standards.

Considering the difference between taste threshold values, taste active value (TAV) is

introduced as an evaluation criterion. TAV was calculated as the ratio of the concentration and the taste threshold in water to compare their contribution to flavor(Schlichtherlecerny & Amadò, 2002).

The umami amino acids in control and treat 1 was little higher than treat 2 (61.4% and 61.51%), while the sufu added with wheat gluten contained higher TAV than control (Table 5). The glutamate has a much higher TAV than aspartate, which indicated that glutamates occupy an important position in sufu taste. Although the content of bitter amino acid in sufu added with the wheat gluten are little higher than that of the traditional sufu, whereas they did not taste bitter. It may be due to the killing phenomenon that masks or suppresses the presentation of the bitter taste. This showed that the interactions between taste substances have a complex and important influence on the overall taste of the food(Lioe, et al., 2005).

Free amino acids are among the main taste substances in foods. Whereas, the results indicated that adding wheat gluten to the traditional sufu might improve its relish, particularly the umami flavor.

Table 5: Amino acid TAV of 3 kinds of sufu

Taste*	Amino acid	Threshold in water (mg/L)**	Taste Active Value (TAV)***		
			Control	Treat1	Treat2
umami	Asp	1000	0.069	0.076	0.073
	Glu	300	1.848	2.959	2.802
	Total TAV		1.917	3.035	2.875
	Content %		61.400	61.510	53.480
Sweet	Ser	1500	0.014	0.025	0.028
	Ala	600	0.060	0.063	0.081
	Thr	2600	0.010	0.013	0.016
	Gly	1300	0.012	0.021	0.026
	Total TAV		0.096	0.122	0.151
	Content %		9.700	8.700	9.700
Bitter	Arg	500	0.004	0.004	0.003
	His	200	0.094	0.125	0.157
	Ile	900	0.052	0.101	0.094
	Leu	1900	0.031	0.048	0.065
	Phe	900	0.050	0.091	0.116
	Met	300	0.036	0.056	0.079
	Val	400	0.087	0.125	0.167
	Total TAV		0.354	0.55	0.681
	Content %		21.370	21.020	25.670

*(KIM, et al., 2003; Salles, et al., 2000)

** (ToshihideNishimura & HiromichiKato, 1988)

***TVA=concentration/taste threshold in water

3.4 Volatile components in sufu samples

The composition and content of volatile flavor components of sufu products were significantly different. The relative contents of volatile components are shown in Figure 9. Among the 38 common compounds, most of them belonged to alcohols (4) and esters (18). The remaining included acids (3), ketones (4), aldehydes (4), hydrocarbons (2) and miscellaneous (3). The results were obtained by a SPME-GC-MS instrument.

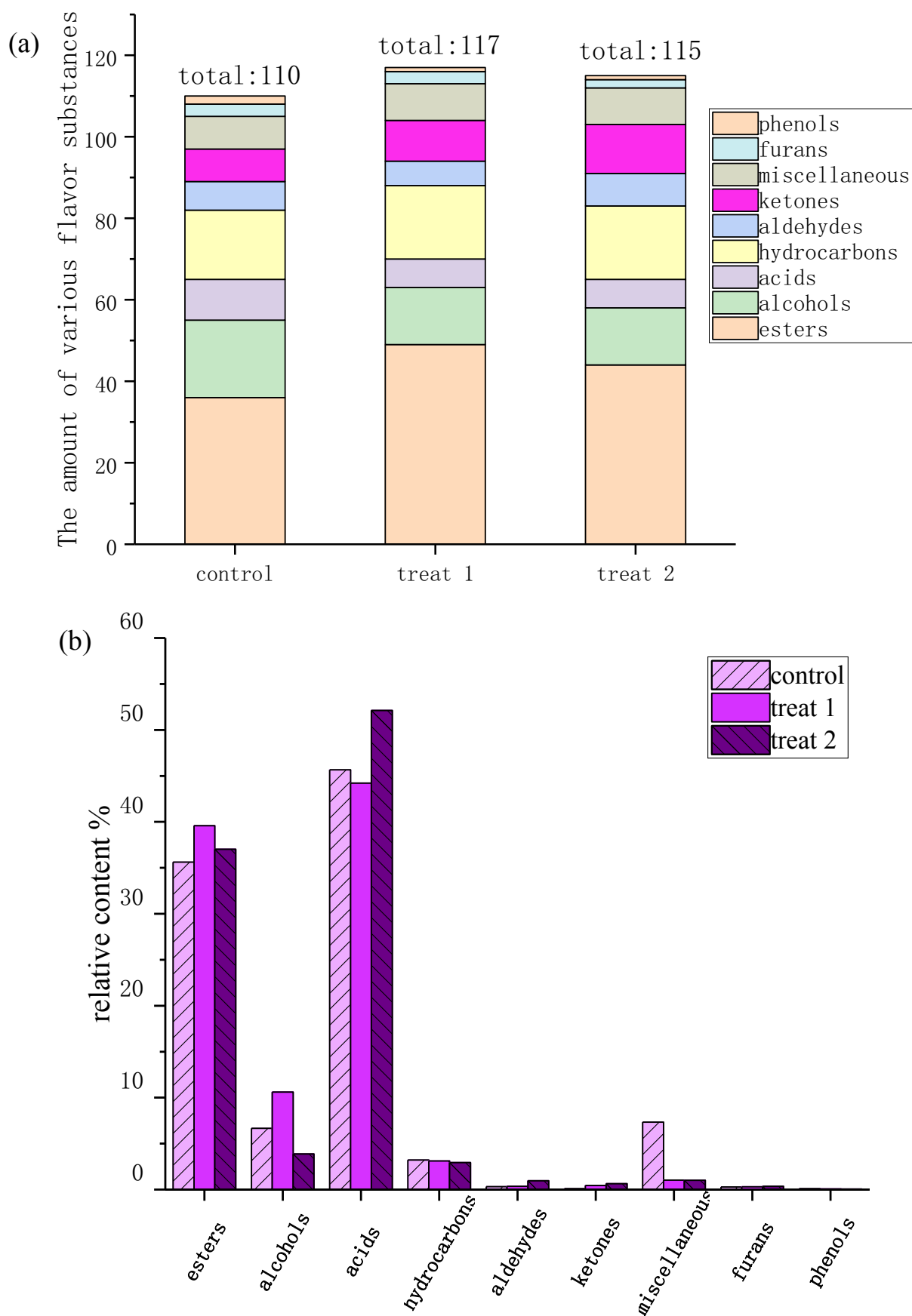


Figure 9: (a) Number of species and total number of volatile components in three kinds of sufu (b) Relative content of each species of volatile flavor components in three kinds of sufu

A combined total of 77 esters were detected, which was the largest group, whereas eighteen esters were common among all the samples. Compared with Chung's research (Chung, 1999), there were little acids detected in the volatile components, the presence of a large number of various esters may indicate that a large amount of acid may be present in the early stages of the production process. Then they reacted with alcohols increasingly and finally form ethyl esters. Simultaneously, fungal lipases hydrolyzed lipids, which intends large amounts of high molecular weight fatty acid esters, such as ethyl 9,12,15-octadecatrienoate and 6,11-eicosadienoic acid, methyl ester. Although usually the threshold of these high molecular weight esters was larger than that of small molecular weight esters and their concentrations were much higher than the reported (Siek, et al., 1971).

Sulfur-containing compounds are essential for the aroma of foods. Common sulfur-containing compounds like 3-(methylthio) propanoic acid ethyl ester and ethanethioic acid, S-(2-methylbutyl) ester were also detected. That is in agreement with the investigation of Hwan et al. (Hwan & Chou, 2010) and Moy et al. (Moy, et al., 2012), both of them have detected sulfur-containing compounds, but differed with Li Tong et al. (T. Li, Zhang, Ling, & Shao, 2013). That may be linked with extraction needle properties or extraction temperature.

Differences in both quantitative and qualitative analysis were also found between sufu products, the compounds including (SS)- or (RR)-4-methyl-2,3-pentanediol, 1-Octen-3-ol, Pentadecanoic acid, ethyl ester and ethyl tridecanoate were found in sufu added the wheat gluten while these compounds were absent traditional.

3.5 Electronic nose analyzes

Principal component analysis (PCA) was performed on the sufu products for the overall aroma components using electronic nose technology (Figure 10). PCA analysis can reduce the variation between multiple variables to fewer variables through

dimensionality reduction and ensuring all original variable information is complete(Eugenia, et al., 2008).

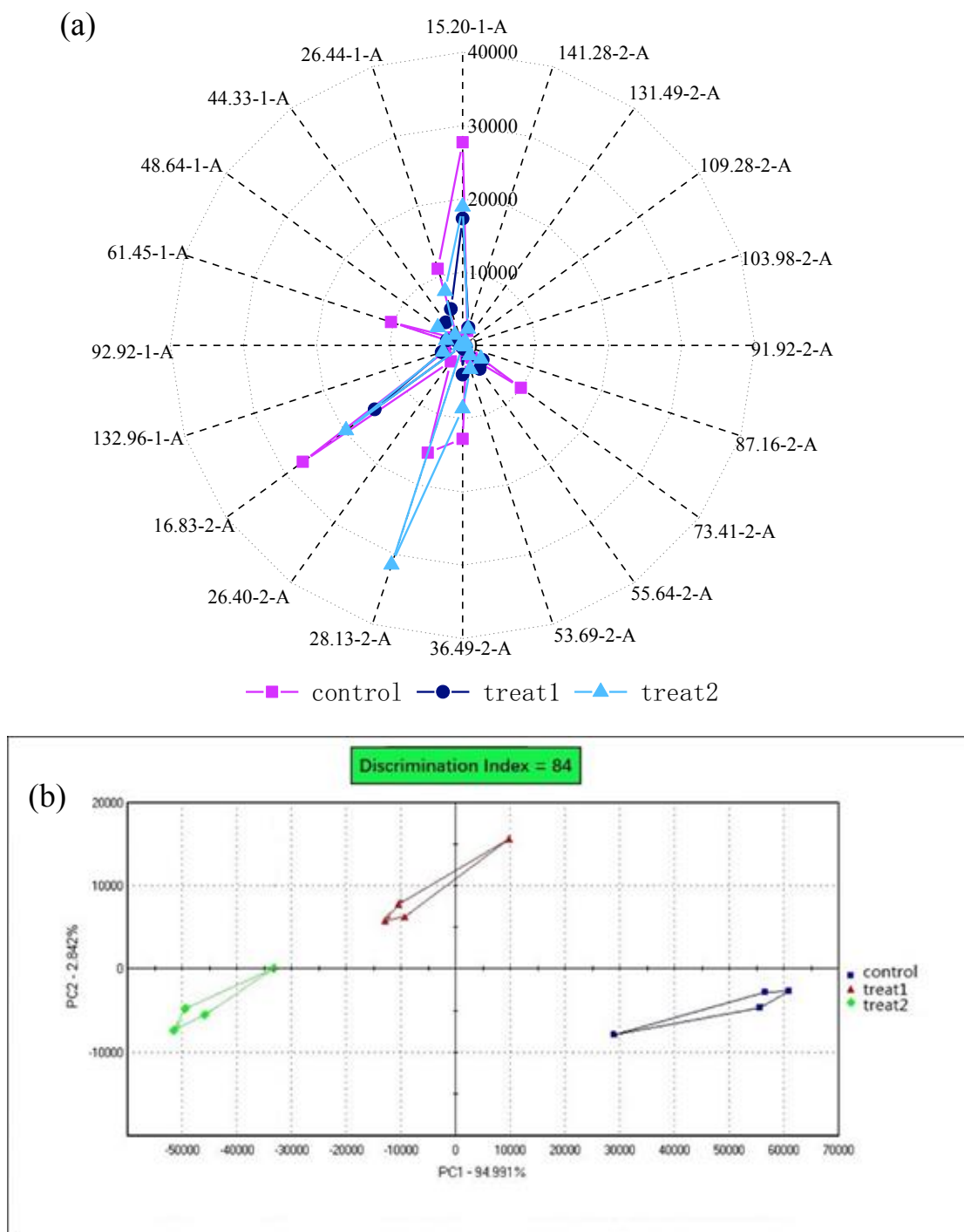


Figure 10: (a) The radar map of fermented bean curd flavor, shown the response loudness of various flavor components on different detectors. Obtained by a E-nose (b)Principal component analysis based on different sufu ($3 \times 4 = 12$ samples)

Analysis of variance showed that the PCA significantly discriminated ($P < 0.05$) sufu products, accounting for a first principal component (PC1) was 94.99% and the second principal component (PC2) is 2.84%, whereas cumulative variations were of 97.83% (Figure 4b). The repeatability results of PCA analysis, electronic nose detection values and volatile aroma components were relatively concentrated with good orientation. The samples were distributed in the second, third and fourth quadrants of the coordinate axis, and can be clearly distinguished.

SPME-GC-MS technology can detect and compare the specific types and contents of volatile flavor substances in the sample but cannot analyze the contribution of these substances as a whole to the flavor characteristics of the sample. The electronic nose, on the other hand, cannot measure the qualitative or quantitative of each volatile substance in the sample, but the overall flavor information of the sample, so the combination of these two is conducive to simultaneously observe the sample flavor from the macroscopic and microscopic perspectives. Thus in the results it can be deduced that the detection results of both the electronic nose technology and SPME-GC-MS technology were echo each other.

3.6 Molecular weight distribution of peptides in sufu

Nowadays, peptides gained more attention by researchers due to their potential functional and nutritional properties. During sufu fermentation, the proteins were broken down and the value of peptides and free amino acids were increased. Molecular weight is an important parameter for the degree of protein hydrolysis, and it is also related to the biological activity of protein hydrolysates, in particular, physiologically active peptides such as soybean peptides in soybean fermented products (Ding, et al., 2017; Singh, et al., 2014). At the same time, the peptide is also an important taste substance. The small peptide with a relative molecular mass of less than 1000 Da has a strong umami taste, and the N-terminal glutamic acid has a stronger umami taste (Taniguchi, et al., 2019).

Molecular weight distribution of the peptide in sufu products were determined and shown in Figure 11.

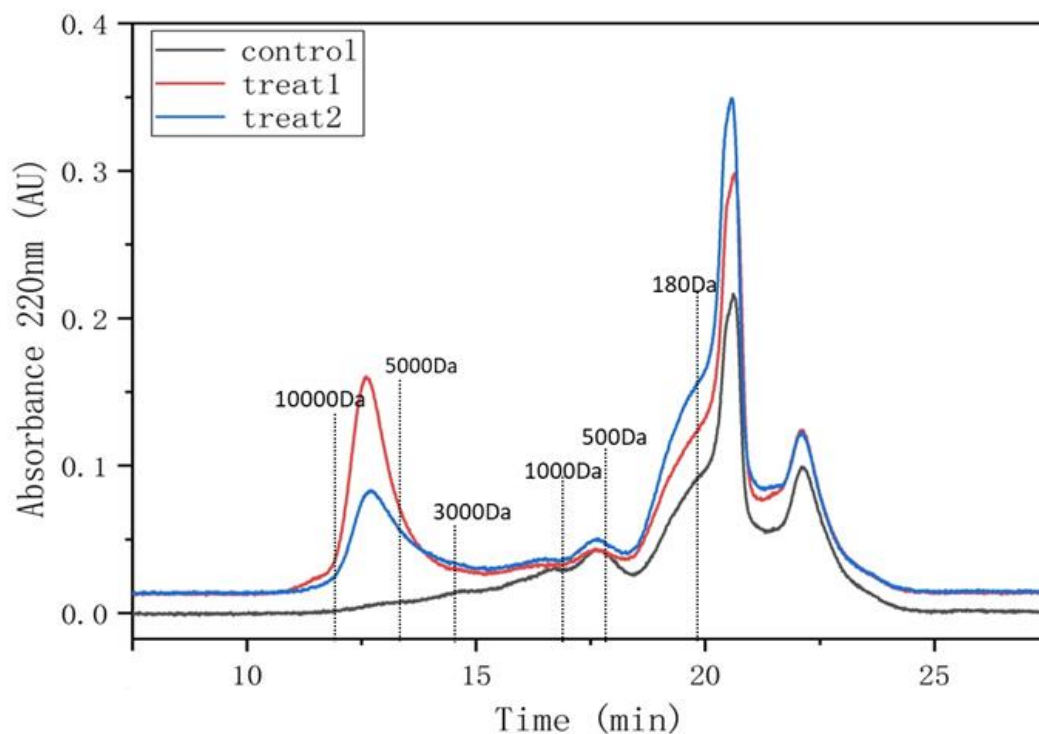


Figure 11: Liquid chromatogram of peptide molecular weight distribution of three kinds of sufu (HPLC-UV with external standards)

Sufu added with wheat gluten showed an increase in the content of small molecules with molecular weight of less than 10,000 Da and most of them are composed free amino acids, accumulative account of 67%-82% of total peptides. Where is interesting is that a peak near the molecular weight of 5000 Da appeared in sufu samples added with wheat gluten but didn't appeared in the traditional sufu. That probably caused by the hydrolysates of wheat gluten protein. A significant increase in peptides, particularly peptides having a molecular weight below 3000 Da, might increase the biological activity of sufu(J.-s. Wang, et al., 2007). The result of distribution of peptides is consistent with the results of the free amino acid test.

3.7 Texture profile analysis

Compared with the texture of the pethze, the hardness and chewiness of the pehtze was decreased, and the other texture indexes increased, which is consistent with the results in the existing literature (W. Liu, et al., 2014; Xia, et al., 2014). The main reason is the role of mucorase in the fermentation process. Compared with the traditional sufu, products added with wheat gluten had lower hardness and chewiness, increased adhesiveness, and no significant differences in cohesion and elasticity (Table 6). The texture of fermented bean curd with added wheat gluten differed significantly from that of traditional fermented bean curd. Lower hardness may cause damage to the block during transport and affect product quality. Therefore, further study is warranted to improve their texture property in standardized manufacturing conditions.

Table 6: Effect of adding wheat gluten on sufu's quality and structure parameters

	hardness	adhesiveness	springiness	chewiness	cohesiveness
control	199.86±9.29 ^{a*}	49.24±4.86 ^a	0.611±0.031 ^a	48.72±6.07 ^a	0.399±0.026 ^a
treat1	134.93±19.97 ^b	87.45±7.40 ^{ab}	0.639±0.049 ^a	35.26±8.29 ^b	0.409±0.013 ^a
treat2	102.04±17.16 ^b	62.39±37.82 ^b	0.594±0.027 ^a	25.63±2.87 ^b	0.423±0.007 ^a

Tested by a TA-XT plus analyzer with a cylindrical probe (diameter 50 mm).

*Values were expressed as mean ± standard deviation.

*Values in the same fraction with different letters (a-c) within a same column differ significantly ($P < 0.05$).

4 Conclusion

The sufu with wheat gluten added were manufactured and analyzed for their physicochemical and taste components characteristics. The result showed that obtained products had higher free amino acids content especially in umami amino acids and more peptides content. This not only improves the umami taste of the product, but also enhances its nutritional value to some extent. Moreover, new type of developed sufu showed a better surface color and richer volatile flavors. For the texture, adding wheat gluten improves sufu's adhesiveness and springiness, but its hardness and chewiness were decreased. On the one hand, these textures let the product easier to spread to food, which is more acceptable to consumers. On the other hand, that can be challenge for transport to keep the whole block. Overall, this study broadens the application of wheat gluten and could lead to the mass production of sufu for commercial purposes.

Future aspect: 1. In this experiment, the effect of gluten on fermented bean curd was studied. The effects of glutenin and gliadin on fermented bean curd can be studied in the future.

2. The addition of wheat gluten reduces the hardness of the fermented bean curd. On the one hand, it enhances the spread ability. On the other hand, it poses a challenge to the block shape keeping during transportation. Therefore, it is possible to conduct subsequent research and expect to improve its hardness.

3. In this experiment, the effects of adding wheat gluten on fermented bean curd and fermented bean curd were studied separately. However, the changes of chemical components and nutrients in the fermented bean curd fermentation process were not studied and can be further studied to explore its fermentation mechanism.

5 Acknowledgements

The present work was supported by the National Natural Science Foundation of China (31872905) and Sub-project of the 13th Five-Year National Key R&D Program (2018YFD 0400604)

6 Reference

- Cao, L. (2014). *Study on the main of Nutrition of Fermented Sufu during Fermentation by Rhizopus oligosporus*. Arggricultural University of Eastnorth, Haerbin.
- Chen, A. (2010). Studies of Water Soluble Wheat Gluten on the Emulsification Stabilization of Milk Beverage (In Chinese). *FOOD RESEARCH AND DEVELOPMENT*, 31(12), 33-36.
- Cheng, L., & Zhao, S. (2005). Function compounds in fermented beancurd (In Chinese). *CHINA CONDIMENT*(12), 10-13.
- Cheng, Y. Q., Hu, Q., Li, L. T., Sarto, M., & Yin, L. J. (2009). Production of Sufu a Traditional Chinese Fermented Soybean Food by Fermentation with *Mucor Flavus*. *Food Science and Technology Research*, 15(4), 347-352.
- Chung, H. Y. (1999). Volatile components in fermented soybean (*Glycine max*) curds. *Journal of Agricultural & Food Chemistry*, 47(7), 2690-2696.
- Ding, Y., Li, X., & Kan, J. (2017). Isolation and identification of flavor peptides from douchi (traditional Chinese soybean food). *International Journal of Food Properties*, 20, 1-13.
- Dou, J., Sun, J., & Lu, F. (2004). Evaluation of taste compounds in water-soluble extract of sufu (In Chinese). *CHINA CONDIMENT*(12), 10-12.
- Eugenia, M. M., Martín, N. R., Giacomazza, D., & Bulone, D. (2008). Correlation between rheological properties and limonene release in pectin gels using an electronic nose. *Food Hydrocolloids*, 22(5), 916-924.
- Han, B. Z., Cao, C. F., Rombouts, F. M., & Nout, M. J. R. (2004). Microbial changes during the production of Sufu—a Chinese fermented soybean food. *Food Control*, 15(4), 265-270.
- Han, B. Z., Rombouts, F. M., & Nout, M. J. R. (2001). A Chinese fermented soybean food. *International Journal of Food Microbiology*, 65(1), 1-10.
- He, B., Wang, S., & Wang, Q. (1986). Changes of protein amino acid composition before and after fermentation of mold tofu (In Chinese). *Amino Acids & Biotic Resources*(2), 10-11.
- Holme, J. (1955). Studies on the physical nature of gliadin. *Cereal Chemistry*, 36, 321-340.
- Hu, H., Yang, D., & Ma, B. (2018). Response Surface Methodology Optimization of Conditions of Thermo-Molded Wheat Gliadin Films Plasticized by Polyethylene Glycol (In Chinese). *Plastics*, 47(1), 72-77.

- Huang, H. L., & Hesseltine, C. W. (1970). Sufu and Lao-Chao. *Journal of Agricultural & Food Chemistry*, Vol:(18), 572-575.
- Huang, K., Jiang, S., & Lin, J. (2006). Screening Protease of Improving the Emulsifying Properties of Wheat Gluten (In Chinese). *ACADEMIC PERIODICAL OF FARM PRODUCTS PROCESSING*(10), 146-148.
- Huang, X., Yu, S., Han, B., & Chen, J. (2018). Bacterial community succession and metabolite changes during sufu fermentation. *LWT-Food Science and Technology*, 97, 537-545.
- Huang, Y.-H., Lu, T.-J., & Chou, C.-C. (2011). Ripening temperature affects the content and distribution of isoflavones in sufu, a fermented soybean curd. *International Journal of Food Science & Technology*, 46(2), 257-262.
- Hwan, C. H., & Chou, C. C. (2010). Volatile components of the Chinese fermented soya bean curd as affected by the addition of ethanol in ageing solution. *Journal of the Science of Food & Agriculture*, 79(2), 243-248.
- Jiang, L. (2012). *Studies on the Relevance between Physicochemical Properties and Sensory Quality of White Sufu*. South China University of Technology, Guangzhou.
- Jiang, X. (2016). Fermented bean curd is beyond imagination (In Chinese). *Science world*, 1, 14-14.
- Kang, K.-M., & Lee, S.-H. (2013). Changes of antioxidant activity and the isoflavone and free amino acid content of fermented tofu with kimchi ingredients and lactic acid bacteria. *Journal of the Korean Society of Food Science and Nutrition*, 42(1), 96-101.
- KIM, SeungHo, LEE, & KyungAe. (2003). Evaluation of taste compounds in water-soluble extract of a doenjang (soybean paste). *Food Chemistry*, 83(3), 339-342.
- Kong, X., & Zhou, H. (2003). Modification of Wheat Gluten by Deamidation (In Chinese). *FOOD SCIENCE*, 24(12), 47-49.
- Kong, X., Zhou, H., & Feng, Q. (2004). Modification of wheat gluten by deamidation with papain (In Chinese). *SCIENCE AND TECHNOLOGY OF FOOD INDUSTRY*(8), 70-72.
- Li, D., Yang, N., Zhou, X., Jin, Y., Guo, L., Xie, Z., Jin, Z., & Xu, X. (2017). Characterization of acid hydrolysis of granular potato starch under induced electric field. *Food Hydrocolloids*, 71, 198-206.
- Li, P., Luan, G., & Song, Y. (2014). Soymilk-clotting Enzyme Activities in Bran Culture of Wutungkiao Mucor (In Chinese). *Soybean Science*, 33(2), 264-268.

- Li, T., Zhang, X., Ling, S., & Shao, K. (2013). Development of the low-salt and flavor fermented bean curd. *Sciencepaper Online (in Chinese)*.
- Li, X. (2015). On Optimization for Texture Analysis of Blood Tofu from Pig and Duck (In Chinese). *Journal of Southwest China Normal University (Natural Science Edition)*, 40(11), 36-42.
- Li, Y. (1997). The fermented bean curd is a quality food that scientifically uses soybeans (In Chinese). *CHINA BREWING*, 16(4), 1-3.
- Liao, L. (2012). *Study on the Deamidation of Wheat Gluten by Organic Acids Upon Hydrothermal Treatment and Reaction Mechanism*. South China University of Technology, Guangzhou.
- Lioe, H. N., Apriyantono, A., Takara, K., Wada, K., & Yasuda, M. (2005). Umami taste enhancement of MSG/NaCl mixtures by subthreshold L- α -aromatic amino acids. *Journal of Food Science*, 70(7), s401-s405.
- Liu, W., Cao, L. L., Chen, C. Y., Zhong-Xue, Y. E., Wang, L. Q., & University, N. A. (2014). Study on optimizational conditions of fermental prophase and textural analysis in the process of sufu fermented by *Rhizopus oligosporus* Saito. *Science & Technology of Food Industry*, 18.
- Liu, Y., Miao, Z., Guan, W., & Sun, B. (2012). Analysis of organic volatile flavor compounds in fermented stinky tofu using SPME with different fiber coatings. *Molecules*, 17(4), 3708-3722.
- Liu, Z. (2000). *Study on tofu gel*. China Agricultural University, Beijing.
- Lu, F. (2005). *Research on Mechanism of Fermentation, Improvement of Quailty and Mode Identification of Sufu*. China Agricultural University, Beijing.
- Lu, F. (2009). Development of a new kind of processed sufu (In Chinese). *CHINA BREWING*, 28(1), 87-88.
- Ma, L., Wei, H., & Wang, Q. (2015). Application of space mutation strain *Mucor* ZY-3 in sufu production (In Chinese). *China Brewing*, 34(4), 62-65.
- Ma, Y.-L., Wang, J.-H., Cheng, Y.-Q., Yin, L.-J., Liu, X.-N., & Li, L.-T. (2014). Selected Quality Properties and Angiotensin I-Converting Enzyme Inhibitory Activity of Low-Salt Sufu, A New Type of Chinese Fermented Tofu. *International Journal of Food Properties*, 17(9), 2025-2038.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, 31(3), 426-428.
- Moy, Y. S., Lu, T. J., & Chou, C. C. (2012). Volatile components of the enzyme-ripened sufu, a Chinese

- traditional fermented product of soy bean. *Journal of Bioscience & Bioengineering*, 113(2), 196.
- Musso, Y. S., Salgado, P. R., & Mauri, A. N. (2016). Gelatin based films capable of modifying its color against environmental pH changes. *Food Hydrocolloids*, 61, 523-530.
- Nakamoto, M., Otsuka, R., Nishita, Y., Tange, C., Tomida, M., Kato, Y., Imai, T., Sakai, T., Ando, F., & Shimokata, H. (2018). Soy food and isoflavone intake reduces the risk of cognitive impairment in elderly Japanese women. *European Journal of Clinical Nutrition*(1 Suppl), 1.
- Qi, S.-p., & Weng, X.-c. (2008). Volatile Flavor Compounds of Tofu. *Journal of Shanghai University*, 14(1), 100-105.
- Salles, C., Hervé, C., Septier, C., Demaizieres, D., Lesschaeve, I., Issanchou, S., & Quéré, J. L. L. (2000). Evaluation of taste compounds in water-soluble extract of goat cheeses. *Food Chemistry*, 68(4), 429-435.
- Schlichtherlecerny, H., & Amadò, R. (2002). Analysis of taste-active compounds in an enzymatic hydrolysate of deamidated wheat gluten. *J.agric.food Chem*, 50(6), 1515.
- Shi, X., & Fung, D. Y. (2000). Control of foodborne pathogens during sufu fermentation and aging. *Critical reviews in food science and nutrition*, 40(5), 399-425.
- Shibasaki, K., & Hessbltine, C. W. (1962). Miso fermentation. *Economic Botany*, 16(3), 180-195.
- Si, J. (2003). *Effect of chemical modification on the adhesion and water resistance of gluten powder as wood adhesive*. Zhengzhou Institute of Technology, Zhengzhou.
- Siek, T. J., Albin, I. A., Sather, L. A., & Lindsay, R. C. (1971). Comparison of Flavor Thresholds of Aliphatic Lactones with Those of Fatty Acids, Esters, Aldehydes, Alcohols, and Ketones and. *Journal of Dairy Science*, 54(1), 1-4.
- Singh, B. P., Vij, S., & Hati, S. (2014). Functional significance of bioactive peptides derived from soybean. *Peptides*, 54(2), 171-179.
- Sun, J. H., Sun, B. Y., Liu, L. L., Liu, Y., Yang, Y. F., Liu, Y. Y., & Shi, Y. G. (2014). Analysis of Volatile Components in Kedong Fermented Bean Curd during the Fermentation. *Modern Food Science & Technology*, 30(1), 200-205+114.
- Tang, W., Jiang, S., & Pan, L. (2006). Effect of Chemical Modification on Digestibility in Vitro and Lysine Validity of Wheat Gluten Protein (In Chinese). *TRANSACTIONS OF THE CHINESE SOCIETY FOR AGRICULTURAL MACHINERY*, 37(8), 29-32.

- Taniguchi, M., Aida, R., Saito, K., Ochiai, A., Takesono, S., Saitoh, E., & Tanaka, T. (2019). Identification and characterization of multifunctional cationic peptides from traditional Japanese fermented soybean Natto extracts. *Journal of Bioscience and Bioengineering*, 127(4), 472-478.
- Tong, Q., Zhu, G., & Gu, Z. (2004). Compound Modification of Wheat Gluten by Acylation and Phosphorylation (In Chinese). *JOURNAL OF THE CHINESE CEREALS AND OILS ASSOCIATION*, 19(6), 11-13.
- ToshihideNishimura, & HiromichiKato. (1988). Taste of free amino acids and peptides. *Food Reviews International*, 4(2), 175-194.
- Wall, J. S., Friedman, M., Krull, L. H., Cavins, J. F., & Beckwith, A. C. (2010). Chemical modification of wheat gluten proteins and related model systems. *Journal of Polymer Science Polymer Symposia*, 24(1), 147-161.
- Wan, S. P., Wang, J. M., Jia, L., Zhang, Y. Y., & Lu, X. D. (2011). Utilization of P. Camemberti in Sufu Production in Comparison with Traditional Sufu Strains. *Advanced Materials Research*, 204-210, 1143-1146.
- Wang, J.-s., Zhao, M.-m., Zhao, Q.-z., & Jiang, Y.-m. (2007). Antioxidant properties of papain hydrolysates of wheat gluten in different oxidation systems. *Food Chemistry*, 101(4), 1658-1663.
- Wang, J., Wang, C., & Zhou, Z. (2006). The Study on the Texture of Microbial Transglutaminase Tofu (In Chinese). *GRAIN PROCESSING*, 31(3), 77-80.
- Wang, L. (2016). *Study on the taste regularity of umami peptide and the preperation of flavor condiments from wheat gluten*. Jiangnan University, Wuxi.
- Wang, L., Du, F., & Shi, J. (2006). Application of Wheat Gluten in Waterborne Interior Emulsion Coatings (In Chinese). *PAINT & COATINGS INDUSTRY*, 36(12), 59-60.
- Wang, R. (2009). *Chinese fermented bean curd*. Beijing: China Light Industry Press.
- Wang, S. (2000). A Brief Account of the Application of Vital Gluten Meal (In Chinese). *SCIENCE AND TECHNOLOGY OF CEREALS,OILS AND FOODS*, 8(2), 5-7.
- Wang, Y., Li, L., & Wang, J. (2012). Analysis of Volatile Flavor Substances in Traditional Fermented Bean Curd's Production of the Changes (In Chinese). *Sichuan Food and Fermentation*, 48(2), 76-81.

- Wu, C. H., Nakai, S., & Powrie, W. D. (1976). Preparation and properties of acid-solubilized gluten. *Journal of Agricultural & Food Chemistry*, 24(3), 504.
- Xia, X., Li, G., Zheng, J., Ran, C., & Kan, J. (2014). Biochemical, textural and microstructural changes in whole-soya bean cotyledon sufu during fermentation. *International Journal of Food Science & Technology*, 49(8), 1834-1841.
- Xiaomiao Luo, & Wang, R. (2015). Development of Sufu Starter by Multi-strains (In Chinese). *China Condiment*(4), 76-80.
- Xie, C., Zeng, H., Wang, C., Xu, Z., & Qin, L. (2018). Volatile Flavor Components, Microbiota and Their Correlations in Different Sufu, a Chinese Fermented Soybean Food. *Journal of Applied Microbiology*.
- Xing, Z., & Du, X. (2010). Compound Modifier Effects on Noodle Quality (In Chinese). *PACKAGING AND FOOD MACHINERY*, 28(2), 27-30.
- Xingda Lu, & Wang, J. (2011). Effect of irradiation on physical and chemical factors of sufu (In Chinese). In *CHINA FOOD ADDITIVES*, vol. z1 (pp. 222-225).
- Xu, L., Yang, N., Wu, F., Jin, Z., & Xu, X. (2017). Effect of acid pretreatment on the physicochemical and antioxidant properties of germinated adlay (*Coix lachryma-jobi* L.). *Journal of Food Processing & Preservation*, 42(1).
- Yan, P., & Yi, J. (2012). Analysis of Volatile Flavor Components in Red Fermented Soybean Curds (In Chinese). *Food Science*(2), 211-215.
- Yan, Z., Bian, K., & Si, J. (2005). Application of Wheat Gluten Powder: A Review (In Chinese). *JOURNAL OF THE CHINESE CEREALS AND OILS ASSOCIATION*, 20(5), 16-20.
- Yin, L. J., Li, L. T., Liu, H., Saito, M., & Tatsumi, E. (2005). Effects of fermentation temperature on the content and composition of isoflavones and beta-glucosidase activity in sufu. *Journal of the Agricultural Chemical Society of Japan*, 69(2), 267-272.
- Yu, R., Tu, X., & Li, J. (2001). The Biochemical Changes in the Culture Phase of Beancurd Cheese (In Chinese). *JOURNAL OF SOUTH CHINA UNIVERSITY OF TECHNOLOGY (Natural Science Edition)*, 29(4), 49-52.
- Yuqing Zhang, Fengfeng Wu, Xueqian Su, Tian Yang, & Xu, X. (2019). Optimization of process conditions for soybean curd rich in wheat gluten. *Food and Fermentation Industries*.
- Zeng, L. (1989). Activated wheat gluten and preparation method (In Chinese). *CEREAL & FEED*

INDUSTRY(2), 8-10.

Zhang, B., Mo, Q., & Chen, S. (2011). Screening of Sufu Mixed Fermentation Strains and Study on Their Fermentation Conditions (In Chinese). *The Food Industry*(7), 15-18.

Zhang, F., & Wu, H. (2012). Application of wheat protein powder in feed (In Chinese). *China Feed*(17), 30-32.

Zhang, H., Zheng, X., & Xi, Y. (2004). Recent Advances on the Research of Wheat Gluten Modification. *JOURNAL OF CHINESE INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY*, 4(1), 93-97.

Zhang, L., & Liu, J. (2002). Elementary introduction on nutritive value of fermented bean curd (In Chinese). *CHINESE CONDIMENT*(5), 9-10.

Zhao, D. (1997). Trivial talk of fermented soybean curd (In Chinese). *CHINA BREWING*, 16(5), 33-34.

Zhao, L., Yin, L., Lei, Z., Bu, Y., Changyun, Z., Ying, Y., & Yanzhuo, K. (2017). Evaluation of Quality Changes of Leisure Dried Tofu During Storage Based on Electronic Nose. *Nanoscience & Nanotechnology Letters*, 9(5), 705-711.

Zhao, X., Liu, L., & Pan, L. (2006). Complex Modification Wheat Gluten and Application in Food (In Chinese). *ACADEMIC PERIODICAL OF FARM PRODUCTS PROCESSING*(10), 118-122.

Zhao, Y., & Zheng, X. (1999). Microbes for the production of bean curd (In Chinses). *CHINESE CONDIMENT*(2), 3-7.

Zhou, J., Lin, Q., & Tang, Q. (2007). The present status of the microbes used in the production proceeding of sufu in China (In Chinese). *FOOD SCIENCE AND TECHNOLOGY* 2007(11), 20-23.

Zhou, S., & Zhong, L. (1986). *Food Science and Food Chemistry (In Chinese)*. Beijing: China Business Press.

Zhu, F. (2016). Production of instant sausage by extruded soybean meal (In Chinese). *Cereal & Feed Industry*, 12(10), 20-25.

Zhu, L., & Cai, X. (2014). Discussion of culture conditions and technology of Mucor sufu. *China Brewing*, 33(10), 123-126.

Zhuang, Y., Tian, P., & Chen, M. (2017). Effects of Different Fermented Bacteria on Flavor of Sufu (In Chinese). *Journal of Hubei University for Nationalities (Natural Sciences Edition)*, 35(3),

270-277.

Zhuang, Y., Tian, P., & Shan, C. (2016). Nutritional Value and Physiological Activity of Sufu (In Chinese). *Journal of Hubei University for Nationalities (Natural Sciences Edition)* , 34(2), 179-183.